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L4: Entry 1 of 1 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287769 B1
TITLE: Method of amplifying DNA fragment, apparatus for amplifying DNA fragment, method of assaying microorganisms, method of analyzing microorganisms and method of assaying contaminant

BSPR:
In this case, a band having a luminous intensity less than the threshold is that of a DNA fragment having low amplification efficiency and low reproducibility. The band having the luminous intensity exceeding the threshold is that of a DNA fragment having high amplification efficiency and high reproducibility. Thus, only the DNA fragment having high amplification efficiency and high reproducibility can be analyzed by employing the band having the luminous intensity exceeding the threshold. Thus, reliability of information obtained by analysis is improved.

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l2 same threshold$ same exceed$	1

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IBM Technical Disclosure Bulletins

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Search History

Today's Date: 10/9/2001

DB Name	Query	Hit Count	Set Name
USPT	l2 same threshold$ same exceed$	1	L4
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USPT	l1 same (nucleic or DNA or RNA or oligo$)	2714	L2
USPT	amplif$ same efficien$	16180	L1

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L3: Entry 1 of 2 File: USPT Nov 7, 2000

DOCUMENT-IDENTIFIER: US 6143496 A
TITLE: Method of sampling, amplifying and quantifying segment of nucleic acid, polymerase chain reaction assembly having nanoliter-sized sample chambers, and method of filling assembly

DEPR:
The data also shows that single DNA molecules or segments can be detected with the "TaqMan" system when PCRs are confined to volumes of 100 nanoliters or less, preferably 60 nanoliters or less, by using capillaries with small diameters and relying on the fortuitously slow rate of diffusion. Many PCR reactions with single molecule sensitivity can be performed simultaneously in small spaces by confining PCR's to small regions in 3 dimensions as described in other embodiments of the present invention. The devices of the invention can be used to measure the number of template molecules in a sample simply by counting the number of positive reactions in replicate PCRs containing terminal dilutions of sample. Due to the closed system environment which prevents carryover contamination, and the ability to automate fluorescence detection, devices according to the present invention and methods for using the devices have significant potential for clinical uses of PCR. An assay based on presence versus absence of PCF product in replicate reactions may be more robust with respect to small changes in amplification efficiency than quantitative competitive assays or time-to-reach-threshold level assays that require assumptions about relative or absolute amplification rates.

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SO JOURNAL OF VIROLOGICAL METHODS, (2000 Mar) 85 (1-2) 75-82.
Journal code: HQR; 8005839. ISSN: 0166-0934.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200005
ED Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000509
AB The laboratory diagnosis of hepatitis B virus (HBV) infection is based mainly on serological assays. Yet the detection and quantitation of viral **DNA** is necessary when addressing directly the question of infectivity or when monitoring the viral load during therapy. Standard hybridization assays allow for exact quantitation, but their sensitivity is limited to 10(5)-10(6) viral genomes per ml of serum. The most sensitive tests for HBV **DNA** are nested PCR systems, which recognize virtually one molecule of the target **DNA** per reaction. However, these assays only provide very coarse quantitative statements. To take **advantage** of both methods, a new assay for HBV **DNA** is described based on the commercial TaqMan system. This assay is capable of quantifying HBV **DNA** from the theoretical lower limit up to 10(10) genome equivalents per ml of serum and, thus, covers the complete range of naturally occurring states of infections. The method was calibrated on the basis of serial plasmid **dilutions** and compared with a well-established nested PCR system. More than 100 HBV positive sera and serial **dilutions** of the Eurohep standard for both ad and ay subtypes were analyzed. The assay reliably detected all HBV positive samples. It shows minimal run-to-run deviations, allows for quantitation that covers eight orders of magnitude, and finally, completely avoids the risk of cross-contamination by PCR products. Thus, this technique combines the se

OF 6 USPATFULL
AN 2001:71314 USPATFULL
TI PCR method for nucleic acid quantification utilizing second or third order rate constants
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PA University of Utah Research Foundation, Salt Lake City, UT, United States (U.S. corporation)
PI US 6232079 B1 20010515
AI US 2000-635344 20000809 (9)
RLI Division of Ser. No. US 1997-869276, filed on 4 Jun 1997
Continuation-in-part of Ser. No. US 1997-818267, filed on 17 Mar 1997
Continuation-in-part of Ser. No. US 1996-658993, filed on 4 Jun 1996, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.
LREP Barnes & Thornburg
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 71 Drawing Figure(s); 52 Drawing Page(s)
LN.CNT 3328
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
IN **Wittwer, Carl T.**, Salt Lake City, UT, United States
DETD . . . amplifies well. The slower rate of probe displacement with an exo.sup.- polymerase apparently requires more time at 60.degree. C. for **efficient amplification** than the exo.sup.+ polymerase. The time required by exo.sup.- polymerases can be reduced by slowly increasing the temperature from 60.degree.. . .
DETD . . . 70.degree. C. to 94.degree. C. The melting curves were converted to melting peaks and displayed (FIG. 44). Note that the **amplification efficiency** of the CFTR fragment appears greater than the neu fragment. The **amplification efficiency** can be rigorously determined by integrating the melting peak data as in Example 16.
DETD . . . patients infected with HIV or hepatitis C is important in prognosis and therapy. Using a control template and monitoring the **efficiency** of **amplification** of both control and natural templates during amplification, accurate quantification of initial template copy number is achieved.
DETD 2. Maximizing the **amplification efficiency** by ensuring adequate time for primer annealing each cycle while:
DETD 3. Maximizing the **amplification efficiency** by ensuring adequate time for product extension each cycle while:
DETD 4. Initiating thermal cycling changes dependent on the level of fluorescence obtained or the current **efficiency** of **amplification**. For example, over-amplification and nonspecific reaction products can be minimized by terminating thermal cycling when the efficiency drops to a. . .
DETD . . . denaturation, annealing, and extension), a change in fluorescence over temperature (product or probe Tm), or a change in extent of **amplification** (**amplification** yield and **efficiency**). These rates, Tm's and their first and second derivatives are used to determine optimal reaction parameters that include denaturation temperature. . .
DETD . . . dyes are used for the control of denaturation, control of extension, and to initiate thermal cycling changes at a certain **amplification** level or **efficiency**. Resonance energy transfer dyes are used for the control of annealing as will be described after the following example.
DETD . . . primers is 3'-labeled with Cy5, no extension can occur.

However, if labeled primer (1-10%) is mixed with unlabeled primer (90-99%), **amplification efficiency** will be slightly decreased, but annealing is observable as fluorescence energy transfer from a double-strand-specific dye to Cy5. The primer. . .